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Intensified naphthoquinone biosynthesis in *Rindera greaca* transgenic roots cultured with hybrid PLA-chitosan scaffolds

Szymon Bober[1*], Kamil Wierzchowski[1], Aleksandra Bandzerewicz[2], Agnieszka Gadomska-Gajadhur[2], Katarzyna Sykłowska-Baranek[3], Maciej Pilarek[1]

[1] *Faculty of Chemical and Process Engineering, Warsaw University of Technology, Waryńskiego 1, 00-645 Warsaw, Poland*
[2] *Faculty of Chemistry, Warsaw University of Technology, 00-664 Warsaw, Poland*
[3] *Faculty of Pharmacy, Medical University of Warsaw, Banacha 1, 02-097 Warsaw, Poland*

**e-mail: szymon.bober.dokt@pw.edu.pl*

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Plants show great potential as a source of biochemical compounds worldwide. They are characterized by high biodiversity, which offers potentially enormous quantities of biologically active substances with a wide spectrum of applications in the pharmaceutical industry. The usage of in vitro methods for plant biomass culturing enhances the productivity of secondary metabolites in comparison to wild plants. Moreover, specific bioengineering techniques can be used to improve biomass proliferation and the desired metabolite biosynthesis. The hybrid polymer–elicitor scaffolds can be a promising tool to increase the production of secondary metabolites, due to the simultaneous utilization of plant biomass immobilization and elicitation. The study aimed to investigate the impact of chitosan characteristics on the naphthoquinone biosynthesis and biomass proliferation of *Rindera graeca* transgenic roots.

The hairy roots were placed on the top of hybrid scaffolds made of polylactic acid (PLA) blended with chitosan to conduct the cultures. The elicitor used in this study was squid and fungi origin. In the case of fungal chitosan, three chitosan molecule masses, i.e., 30 kDa, 300 kDa, and 3000 kDa, and different viscosities, i.e., 10-120 cps, 100-300 cps, and 2000-3500 cps, were used. The average concentration of the polysaccharide obtained in the used constructs was around 25%$_{m/m}$. Transgenic roots immobilized on PLA scaffolds without chitosan were used as a control system. The growth of the biomass and the produced naphthoquinones in *Rindera graeca* transgenic root cultures were determined quantitatively.

The squid chitosan causes intensified hairy root proliferation compared to the fungal chitosan and the control system. However, the biomass cultures with fungal chitosan produced significantly more naphthoquinones than cultures containing polymeric scaffolds modified with squid chitosan. Along with the increase in the mass of the chitosan molecule, the increase in the plant biomass was noticed. The highest naphthoquinone concentration was noticed for scaffolds containing 300 kDa chitosan. The chitosan viscosity had no notable effect on biomass proliferation and the production of naphthoquinones.

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